

Highlights of CRISPR-Cas9 Genome Editing

Ali Movahedi¹
Amir Almasizadehyaghuti¹
Kourosh Mohammadi¹
Sirus Sangari¹
Qiang Zhuge¹

¹Assistant Professor, Nanjing Forestry University, the Cooperative Innovation Center of Southern Modern Forestry, Nanjing, Jiangsu, China

Abstract

The advantage of CRISPR genome editing is not limited to only its cost, but it is easy to use in any lab with molecular biology expertise. On the other hand, CRISPR does not need to protein engineering for targeting each gene, while TALE and ZF nucleases are needed. CRISPR-Cas9 genome editing system needs only a DNA construct to encode the target specific gRNA and Cas9 in one expression vector. Furthermore, CRISPR-Cas9 can edit multiple genome with homolog sequencing simultaneously, resulting in increasing the efficiency.

Keywords

CRISPR; Genome editing

Introduction

Trans-activating crRNA (tracrRNA) is one motif that is necessary to recruit the Cas9 nuclease complex and direct it to the target sequence, using the guide RNA (gRNA) including target recognition sequence. In addition, one CRISPR-Cas9 needs to detect target with the Protospacer Adjacent Motif (PAM), which is defined as NGG or etc. CRISPR-Cas9 enables to excise 3 to 4 nucleotides of the upstream of the PAM motif.

On the other hand, inside the cells there exist some repair pathways for DNA, which will be activated with Double-stranded breaks (DSB) on target sequencing. In CRISPR-Cas9 genome editing there are two ways for starting edition: 1) Non-Homologous End Joining (NHEJ) that enables to insert or delete nucleotides or fragment randomly, at the site of repair on target sequencing, leading to suppress the gene with no longer expression and cause to knock-out. 2) The Homologous Recombination (HR) sequences cause to integrate DNA or knock-in to the DSB sites, leading to make precise mutation on the target genes.

TALENs, ZFNs and CRISPR-Cas9

There are some differences and advantages between TALENs (transcription activator-like effector nucleases), ZFNs (zinc finger nucleases) and CRISPR-Cas9, which are the best double-stranded DNA break genome editing technologies. Regarding with the targets, TALENs will target both proteins and DNAs such as ZFNs, but unlike CRISPR-Cas9 that target only DNAs using one guide RNA (gRNA). In addition, regarding to the construct of them shows ZFNs bind to the motifs in a $\beta\beta\alpha$ configurations and also, detect 3 bp of DNA α -helix region. TALENs recognize the specific base pair sequences of the DNA, whereas CRISPR-Cas9 that recognize target only with 20 nucleotides crRNA (CRISPR RNA) attached to tracrRNA (Transcription CRISPR RNA) and Cas9 endonucleases to detect specific base pair sequences, which is termed PAM. Although, TALENs and ZFNs need to customize protein for editing each gene sequences, CRISPR-Cas9 needs only one expression vector including gRNA-Cas9 construct, and enables to multigenes editing simultaneously. Purification of genomic DNA is necessary to investigate of chromosomes including chromatin-associated RNAs and proteins. According to the Gilbert et al., modification of CRISPR-Cas9 allows us to edit genome in multiple targets [1]. This is possible to add one trackable tag and one molecular localization signal (NLS) into inactive or dead Cas9 (dCas9) to create a bind between proteins and DNAs for targeting by guide RNA (gRNA) [2]. Using of databases is required to design gRNA for targeting genes and avoiding of increasing off-targets.

CRISPR-Cas9 and epigenetic

Epigenetic modifications have been always to play very important roles in biological processes via influence on genomic DNA at specific loci and histone proteins. Cytosine methylation and acetylation are most important of epigenetic modification that affect on gene expression. These days, CRISPR-Cas9 is considered to genome editing for these epigenetic spots.

Article Information

DOI: 10.31021/mmmb.20181101
Article Type: Editorial
Journal Type: Open Access
Volume: 1 Issue: 1
Manuscript ID: MMBB-1-101
Publisher: Boffin Access Limited

Received Date: October 17, 2017
Accepted Date: December 24, 2017
Published Date: January 02, 2018

Citation: Movahedi A, Almasizadehyaghuti A, Mohammadi K, Sangari S, Zhuge Q. Highlights of CRISPR-Cas9 Genome Editing. Methods Microbiol Mol Biol. 2018 Jan;1(1):101

*Corresponding author:

Ali Movahedi
Assistant Professor
Nanjing Forestry University
The Cooperative Innovation Center of Southern Modern Forestry
Nanjing, Jiangsu, China
E-mail: ali66469@yahoo.com

Copyright: © 2018 Movahedi A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 international License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Liao et al. reported that presented three active methyltransferases in embryonic stem cells of human, can be knocked-out by CRISPR-Cas9 to indicate usable, capable of giving rise to several different cell types on the DNA methylation [3].

Acknowledgment

We welcome all readers to prepare articles in plant genome editing by CRISPR-Cas9 for submitting to Methods of Microbiology and Molecular Biology journal.

References

1. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*. 2013 Jul;154(2):442-451.
2. Fujita T, Fujita H. Efficient isolation of specific genomic regions and identification of associated proteins by engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP) using CRISPR. *Biochem Biophys Res Commun*. 2013 Sep;439(1):132-136.
3. Liao J, Karnik R, Gu H, Ziller MJ, Clement K, et al. Targeted disruption of DNMT1, DNMT3 and DNMT3B in human embryonic stem cells. *Nat Genet*. 2015 May;47(5):469-478.